

What is claimed is:

1. A process for the preparation of L-threonine using L-threonine-producing bacteria from the family Enterobacteriaceae, wherein
 - 5 a) the bacterium is inoculated into at least a first nutrient medium and cultivated,
 - b) some of the fermentation broth is abstracted, wherein more than 90 vol.% of the total volume of the fermentation broth remains in the fermentation container, then
 - 10 c) the remaining fermentation broth is topped up with one or more further nutrient media, wherein the further nutrient medium or further nutrient media contains at least one source of carbon, at least one source of nitrogen and at least one source of phosphorus, and cultivation is continued under conditions which enable the formation of L-threonine,
 - d) steps b) and c) are optionally performed several times, and
 - 15 e) the concentration of the source(s) of carbon is adjusted to a maximum of 30 g/l during cultivation in accordance with step c) and/or d).
2. A process according to claim 1, wherein cultivation step (a) is performed by the batch process.
- 25 3. A process according to claim 1, wherein cultivation step (a) is performed by the fed batch process, wherein at least one added nutrient medium is used.
4. A process according to claim 1, 2 or 3, wherein less than 8 vol.% of the fermentation broth is abstracted.
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5. A process according to claim 1, 2 or 3, wherein less than 5 vol.% of the fermentation broth is abstracted.
6. A process according to claim 1, 2 or 3, wherein less than 2 vol.% of the fermentation broth is abstracted.
- 5 7. A process according to claim 1, 2 or 3, wherein the L-threonine formed is purified.
8. A process according to claim 1, wherein the source of carbon is one or more compounds chosen from the group saccharose, molasses from sugar beet or sugar cane, 10 fructose, glucose, starch hydrolysate, cellulose hydrolysate, arabinose, maltose, xylose, acetic acid, ethanol and methanol.
9. A process according to claim 1, wherein the source of 15 nitrogen is one or more organic nitrogen-containing substances or substance mixtures chosen from the group peptones, yeast extract, meat extract, malt extract, corn steep liquor, soy bean flour and urea and/or one or more inorganic compounds chosen from the group ammonia, ammonium-containing salts and salts of nitric acid.
- 20 10. A process according to claim 9, wherein the ammonium-containing salts and salts of nitric acid are ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate, ammonium nitrate, potassium nitrate and potassium sodium nitrate.
- 25 11. A process according to claim 1, wherein the source of phosphorus is phosphoric acid or the alkali metal or alkaline earth metal salts or polymers thereof or phytic acid.
- 30 12. A process according to claim 11, wherein the alkali metal salts of phosphoric acid are potassium

dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts.

13. A process according to claim 1, wherein the bacteria from the family Enterobacteriaceae are the species
5 *Escherichia coli*.

14. A process according to claim 1, wherein the bacterium from the family Enterobacteriaceae contain at least one *thrA* gene or allele which codes for a threonine-insensitive, aspartate kinase I - homoserine
10 dehydrogenase I.

15. A process according to claim 1, wherein the bacterium from the family Enterobacteriaceae contains a stop codon chosen from the group opal, ochre and amber, preferably amber, in the *rpoS* gene and a t-RNA
15 suppressor chosen from the group opal suppressor, ochre suppressor and amber suppressor, preferably amber suppressor.

16. A process according to claim 1, wherein steps b) and
20 c) are repeated, in accordance with d), 0 to 100 times, preferably 2 to 80 times, preferably 4 to 50 times and particularly preferably 5 to 30 times.

17. A process according to claim 1, wherein the time between complete abstraction of the fermentation broth down to more than 90 vol.% of the total volume and
25 complete topping up to about 100% with nutrient media is at most 5 hours.

18. A process according to claim 17, wherein complete topping up with nutrient media takes at most 2 hours.

19. A process according to claim 1, wherein, in the fed
30 nutrient medium or fed nutrient media, the phosphorus to carbon ratio (P/C ratio) is adjusted to at most 4; at most 3; at most 2; at most 1.5; at most 1; at most

0.7; at most 0.5; at most 0.48; at most 0.46; at most 0.44; at most 0.42; at most 0.40; at most 0.38; at most 0.36; at most 0.34; at most 0.32; at most 0.30.

20. A process according to claim 1, wherein the withdrawn culture broth is provided with oxygen or an oxygen-containing gas until the concentration of the source of carbon drops to less than 2 g/l; less than 1 g/l; less than 0.5 g/l.
21. A process according to claim 19, wherein the L-threonine formed is purified.
22. A process according to claim 19, wherein at least 90% of the biomass is first removed from the culture withdrawn in step (b) and then at least 90% of the water is removed.
- 15 23. A process according to claim 1, 2 or 3, wherein the concentration of the source of carbon during the culture process is adjusted to at most 20, 10 or 5 g/l.
- 20 24. A process according to claim 1, 2 or 3, wherein the concentration of the source of carbon during the culture process is adjusted to at most 5 or 2 g/l.
25. A process according to claim 1, 2 or 3, wherein the concentration of the source of carbon during the culture process is adjusted to at most 5 g/l.
- 25 26. A process according to claim 1, 2 or 3, wherein the concentration of the source of carbon during the culture process is adjusted to at most 2 g/l.
27. A process according to claim 1, 2 or 3, wherein the yield of L-threonine formed, with respect to the source of carbon used, is at least 31 wt.%.

28. A process according to claim 1, 2 or 3, wherein the yield of L-threonine formed, with respect to the source of carbon used, is at least 37%.
29. A process according to claim 1, 2 or 3, wherein the yield of L-threonine formed, with respect to the source of carbon used, is at least 42%.
30. A process according to claim 1, 2 or 3, wherein the yield of L-threonine formed, with respect to the source of carbon used, is at least 48 wt.%.
- 10 31. A process according to claim 1, 2 or 3, wherein L-threonine is formed with a space-time yield of 5.0 to more than 8.0 g/l per hr.
32. A process according to claim 1, 2 or 3, wherein L-threonine is formed with a space-time yield of 3.5 to more than 5.0 g/l per hr.
- 15 33. A process according to claim 1, 2 or 3, wherein L-threonine is formed with a space-time yield of 2.5 to more than 3.5 g/l per hr.
34. A process according to claim 1, wherein a fed batch process is used in cultivation step a), and that L-threonine is formed with a space-time yield of at least 2.5 to 5.0 g/l per hr.
- 20 35. Saccharose-utilizing transconjugants of *Escherichia coli* K-12 deposited as DSM 16293 at the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany).
- 25 36. A process according to claim 1, 2 or 3, wherein strains are used which have at least the following features:

- a) a threonine-insensitive aspartate kinase I - homoserine dehydrogenase I, which is optionally present overexpressed, and
- 5 b) a stop codon chosen from the group opal, ochre and amber, preferably amber in the rpoS gene and a t-RNA suppressor chosen from the opal suppressor, ochre suppressor and amber suppressor.

37. A process according to claim 1, 2 or 3, wherein
10 strains are used which have at least the following features:

- a) a threonine-insensitive aspartate kinase I - homoserine dehydrogenase I, which is optionally present overexpressed,
- 15 b) are not able to degrade threonine under aerobic conditions,
- c) an at least partial isoleucine requirement, and
- d) growth in the presence of at least 5 g/l of threonine.

20 38. A process according to claim 1, 2 or 3, wherein strains are used which have at least the following features:

- a) a threonine-insensitive aspartate kinase I - homoserine dehydrogenase I, which is optionally present overexpressed,
- 25 b) a stop codon chosen from the group opal, ochre and amber, preferably amber in the rpoS gene and a t-RNA suppressor chosen from the group opal suppressor, ochre suppressor and amber suppressor,

- c) are not able to degrade threonine under aerobic conditions, preferably due to attenuation of threonine dehydrogenase,
- d) an at least partial isoleucine requirement, and
- 5 e) growth in the presence of at least 5 g/l of threonine.

39. A process according to claim 36, 37 or 38, wherein the strain used also contains one or more features chosen from the group

- 10 39.1 attenuation of phosphoenol pyruvate carboxykinase coded by the *pckA* gene,
- 39.2 attenuation of phosphoglucose isomerase coded by the *pgi* gene,
- 15 39.3 attenuation of the *YtfP* gene product coded by open reading frame *ytfP*,
- 39.4 attenuation of the *YjfA* gene product coded by open reading frame *yjfA*,
- 39.5 attenuation of pyruvate oxidase coded by the *poxB* gene,
- 20 39.6 attenuation of the *YjgF* gene product coded by open reading frame *yjgF*,
- 39.7 enhancement of transhydrogenase coded by the genes *pntA* and *pntB*,
- 25 39.8 enhancement of phosphoenolpyruvate synthase coded by the *pps* gene,
- 39.9 enhancement of phosphoenolpyruvate carboxylase coded by the *ppc* gene,

- 39.10 enhancement of regulator RseB coded by the *rseB* gene,
- 39.11 enhancement of galactose proton symporters coded by the *galP* gene,
- 5 39.12 the ability to use saccharose as a source of carbon,
- 39.13 enhancement of the YedA gene product coded by open reading frame *yedA*,
- 10 39.14 growth in the presence of at least 0.1 to 0.5 mM or at least 0.5 bis 1 mM of borrelidin (borrelidin resistance),
- 39.15 growth in the presence of at least 2 to 2.5 g/l or at least 2.5 to 3 g/l of diaminosuccinic acid (diaminosuccinic acid resistance),
- 15 39.16 growth in the presence of at least 30 to 40 mM or at least 40 to 50 mM of α -methylserine (α -methylserine resistance),
- 39.17 growth in the presence of at most 30 mM or at most 40 mM or at most 50 mM of fluoropyruvic acid (fluoropyruvic acid sensitivity),
- 20 39.18 growth in the presence of at least 210 mM or at least 240 mM or at least 270 mM or at least 300 mM of L-Glutamic acid (glutamic acid resistance),
- 25 39.19 an at least partial requirement for methionine,
- 39.20 an at least partial requirement for m-diamino-pimelic acid,
- 39.21 growth in the presence of at least 100 mg/l of rifampicin (rifampicin resistance),

- 39.22 growth in the presence of at least 15 g/l of L-lysine (lysine resistance),
- 39.23 growth in the presence of at least 15 g/l methionine (methionine resistance),
- 5 39.24 growth in the presence of at least 15 g/l of L-aspartic acid (aspartic acid resistance), and
- 39.25 enhancement of pyruvate carboxylase coded by the pyc gene.